The Bacterial Membrane Insertase YidC Is a Functional Monomer and Binds Ribosomes in a Nascent Chain-Dependent Manner

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The synthesis and folding of proteins often occurs in kinetically coupled processes in order to avoid the accumulation of aggregation-prone unfolded polypeptide chains. Chaperones are specifically recruited to the ribosomal tunnel exit where they wait for the appearance of appropriate binding sites on the emerging polypeptide chains in order to ensure early binding of folding factors. For a number of chaperones and nascent chain modifying enzymes, the binding sites on the ribosome were mapped to high molecular resolution and their relevance for cotranslational protein folding has been studied in detail [1].

In contrast, our understanding of how ribosomes associate with the components that mediate protein insertion into lipid bilayers is still relatively scarce. Due to their hydrophobic nature, nascent membrane proteins have a much higher risk to form insoluble aggregates. Hence, a tight coupling of the synthesis and insertion of membrane proteins appears crucial. Two evolutionary conserved systems mediate the membrane integration of proteins: the Sec61/SecY translocon (Sec complex) and protein insertases of the YidC/Oxa1/Alb3 protein family. The Sec complex forms a translocation pore that allows the complete translocation of secretory proteins through the membrane, as well as the insertion of membrane proteins via a lateral gate [2]. Ribosomes can directly dock onto the Sec complex so that the ribosomal polypeptide tunnel and the translocation pore in the membrane form one continuous channel [3,4]. The targeting of ribosomes to the Sec complex is thereby initiated by the signal recognition particle in conjunction with its receptor [5].

Members of the YidC/Oxa1/Alb3 protein family mediate the insertion of proteins into membranes of bacteria, mitochondria and chloroplasts, respectively. The precise mode of action is not known, but their substrates typically lack larger soluble domains on the *trans* side of the membrane, suggesting that their major activity is the integration and folding of transmembrane segments in the lipid bilayer rather than the translocation of hydrophilic segments across the membrane. Nevertheless, the Oxa1 protein of the mitochondrial inner membrane, in cooperation with its homolog Cox18, was shown to facilitate membrane translocation of even long hydrophilic domains [6,7] and electrophysiological measurements with purified Oxa1 showed an ability to form substrate-gated pores [8].

The membrane-embedded insertase domain of bacterial YidC (Fig. 1a), mitochondrial Oxa1 and chloroplast Alb3 are closely related and functionally interchangeable [9–11], but Oxa1, in contrast to YidC, contains a C-terminal ribosome binding domain (Fig. 1b) that connects the protein permanently to mitochondrial ribosomes [12,13]. The permanent tethering of mitochondrial ribosomes to the membrane is presumably a consequence of the evolution of the mitochondrial genome during which genes coding for hydrophilic proteins were lost.

The bacterial YidC protein lacks a C-terminal ribosome binding domain and only exposes a very short C-terminal stretch of 13 residues into the cytosol. Cross-linking studies indicated that, during the synthesis of membrane proteins, YidC and bacterial ribosomes are in close contact [14,15] but it remained unclear whether YidC binds bacterial ribosomes directly. This important issue was now carefully addressed by the group of Arnold Driessen in a study published in this issue of *Journal of Molecular Biology* [16]. By use of fluorescence correlation spectroscopy, the authors showed that YidC does not associate with bacterial ribosomes (Fig. 1b). Interestingly, the addition of just six histidine residues to the C-terminus of YidC is sufficient to bind YidC to ribosomes particularly upon low pH where more of the histidine residues are positively charged. This is a very

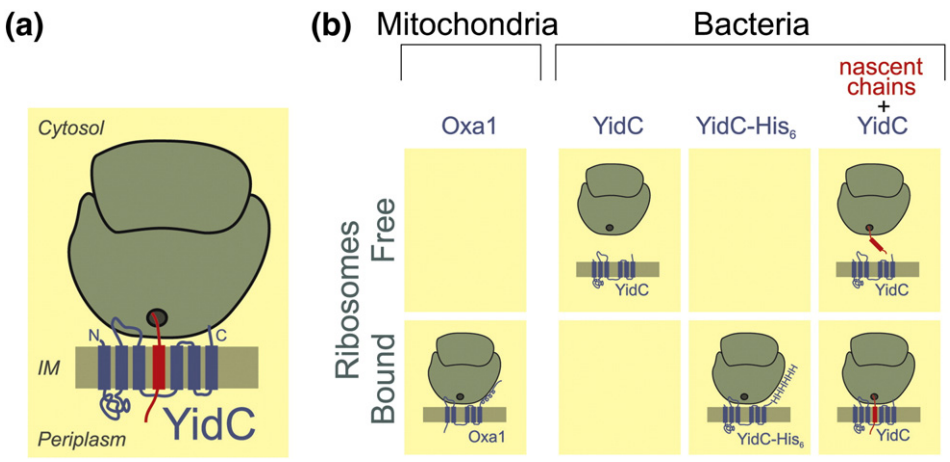

Fig. 1. (a) The inner membrane (IM) protein YidC associates with translation-active ribosomes, presumably via low-affinity interactions of one of its cytosolic loops and its short C-terminus. (b) The mitochondrial Oxa1 protein binds permanently to ribosomes even in the absence of nascent chains. In bacteria, YidC does not associate with ribosomes unless an additional sequence is added to its C-terminus (such as a positively charged hexahistidine tag) or when nascent YidC substrates contribute to the interaction.

interesting observation since histidine-tagged YidC was used before in cryo-electron microscopic studies claiming that YidC, like Oxa1, would bind in proximity to the polypeptide exit tunnel to ribosomes [17]. Nevertheless, Kedrov *et al.* found that, if ribosomes expose a YidC substrate from their exit tunnel, there was a robust interaction with YidC particularly when YidC was reconstituted into nanodiscs to mimic a membrane environment (Fig. 1b). This suggests that bacterial ribosomes do not associate with YidC unless they synthesize YidC substrates. *In vivo*, additional factors, particularly the bacterial signal recognition particle and its receptor, might further contribute to this interaction.

Moreover, Kedrov *et al.* showed that reconstituted YidC is largely monomeric and forms oligomers only when present at very high concentrations. Monomeric YidC binds to the nascent chain-containing ribosomes suggesting that one YidC protein can form the functional unit [16]. This is reminiscent to the recent observations that a monomeric Sec complex represents the functional unit of the translocon [18,19].

It will be very interesting to study the dynamic interaction of ribosomes with the Sec complex and with YidC in living bacteria. It was proposed that the insertion of multispanning inner membrane proteins is initiated by the Sec complex that then passes substrates on to YidC and that ribosomes would trigger the substrate transfer reaction [20,21]. Further studies will certainly be necessary to address this dynamic interplay of ribosomes with the insertion machineries of the bacterial inner membrane.

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